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Development of a novel colorimetric indicator pad for detecting aldehydes

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Abstract

A colorimetric indicator was developed and a colorimetric indicator pad was fabricated for the rapid detection of aldehydes. The detection pad has two sides: an observation side on top and a barrier on the bottom. The top side contains a reagent which reacts directly with aldehydes to produce a color change, while the bottom side is coated with a double-sided plastic tape barrier to prevent the escape of chemicals. Sensitivity of the indicator pads was determined using the vapor sensitive ASTM F739 technique with the presence of the indicator. A significant indicator color change (yellow to red) occurred about 5 min before the infrared analyzer response of the ASTM method. The chemical principle and reaction characterization of the test are described. The stability and potential interferences of the indicator pad were also examined by directly spiking aldehydes and compounds with other functional groups, respectively, onto the indicator pads. The newly developed aldehyde indicator pad should find utility in detecting aldehydes in both liquid and vapor phases and in collecting aldehyde permeation through PPE for further study.
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Keywords: Health and safety; Skin chemical exposure; Development of colorimetric indicator; Aldehyde detector

1. Introduction

Glutaraldehyde is used widely in a variety of industries. In the chemical industry, glutaraldehyde is used as an intermediate agent in pesticide synthesis, to tan soft leathers, and to produce adhesives for electrical products [1]. In the healthcare industry, glutaraldehyde is used as a cold disinfectant, to process X-ray film, and to fix tissues in microscopy [1,2]. Aldehydes are strongly irritating to the nose, eyes, and skin, and can cause allergic contact dermatitis from occasional or incidental occupational exposure [1–3]. The 1995 ACGIH (American Conference of Government Industrial Hygienists) short-term exposure limit (STEL)/ceiling for glutaraldehyde is 0.2 ppm (0.82 mg/m^3), as its recommended exposure limit [4].

Personal protective equipment (PPE), such as chemical-resistant gloves and protective clothing, is routinely employed to prevent skin exposure to toxic chemicals in the workplace [5–7]. Chemical-resistant gloves are typically selected based on manufacturers' recommendations. However, many workplace variables influence glove performance, including flexing, increased temperature, mixtures of two or more chemicals, and differences among glove manufacturers [8–10]. Limited laboratory test data cannot address all these variables [8–11].

There is a pressing need for sensors or indicators that can be worn inside or beneath the PPE barrier to warn the user that a chemical breakthrough has occurred and the user needs to change the PPE. These sensors or indicators would also be valuable training tools for users and provide information to industrial hygienists on proper glove selection. However, very few technologies exist that are amenable to this application. Most methods used for the detection of aldehydes do not have the required sensitivity or are too bulky to wear underneath gloves or protective clothing. Chemically sensitive indicator pads have shown potential for this application [12–17]. Other promising technologies include fiber optic sensors and conducting polymer electrodes sewn into protective clothing [18,19]. Regardless of what detection methodology is used, a chemically sensitive layer is required and additional research in this area is needed.

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Several aldehyde indicator compounds have been developed, using Jones oxidation [Cr(VI)] [20], Schiff's reagent (p-rosanaline hydrochloride) [21], 2,4-dinitrophenylhydrazine reagent [20,22], and sodium sulfite (Serim™ Research Corp, Elkhart, IN and Comply™ 3 M Health Care, St. Paul, MN). These compounds provide aldehyde classification tests in the liquid rather than the vapor phase [20–22]. In addition, chromium(VI) has a history as a cancer suspect agent [23] while 2,4-dinitrophenylhydrazine needs a few minutes to develop a color characteristic in the detection system [20]. On exposure to aldehydes, Schiff's reagent changes color from dark-purple to light-blue, so it is hard to distinguish the color change of the indicator pad in the vapor phase. We report here a new development of a colorimetric indicator to detect and collect glutaraldehyde and alkaline glutaraldehyde in both liquid and vapor phases.

2. Experimental methods

2.1. Chemicals, pad materials, and other apparatuses

Unless otherwise specified, the starting glutaraldehyde, methanol, glycerol, methyl red, alkaline glutaraldehyde solutions, sodium hydroxide, and other solvents used for this study were obtained from a commercial supplier (Aldrich Chemical, Milwaukee, WI) and used as neat standard chemicals without further purification. The pad material (Whatman Benchkote Plus, Catalog No.: 2301-6150) was purchased from Fisher Scientific (Pittsburgh, PA). A Miran-IA (Miniature Infrared Analyzer) closed-loop configuration (Fig. 1), which consisted of a metal bellows pump (Model MB-41, Metal Bellows, Sharon, MA), a 2.5-cm chemical permeation cell (2.5 cm in diameter, AMK Glass Company, Vineland, NJ), and the Miran (Foxboro, Norwalk, CT), was used to evaluate the indicator pad.

2.2. Colorimetric indicator

2.2.1. Aldehyde indicator solution preparation

A magnetic stirring bar, 500 mL of water, and 420 mL of methanol were placed into a 2-L Erlenmeyer flask, and a stirrer immediately started. After stirring for 10 min, 0.35 g of methyl red {2-[4-(dimethylamino) phenylazo]benzoic acid, sodium salt, $(CH_3)_2NC_6H_4N=NC_6H_4CO_2Na$}, was added to the solution with continuous stirring for 1 h. The solution immediately adopted a yellow colored appearance. Glycerine (80 mL) was slowly added into the solution to a final volume of 1 L [a final concentration of $(CH_3)_2NC_6H_4N=NC_6H_4CO_2Na$ is 1.2 mM]. The dissolution process was allowed to proceed at room temperature with continuous stirring for an additional 1 h. Once the solution had become completely homogenous, the color of the solution changed from yellow to orange. Then, pellets of sodium hydroxide were added to the solution to a final concentration of 0.7 mM, and the solution immediately changed from orange to a yellow color. The crude solution was purified by vacuum filtration on the Hirsch funnel, and the filtrate was collected. This filtrate was used as the final indicator solution for making the indicator pads.

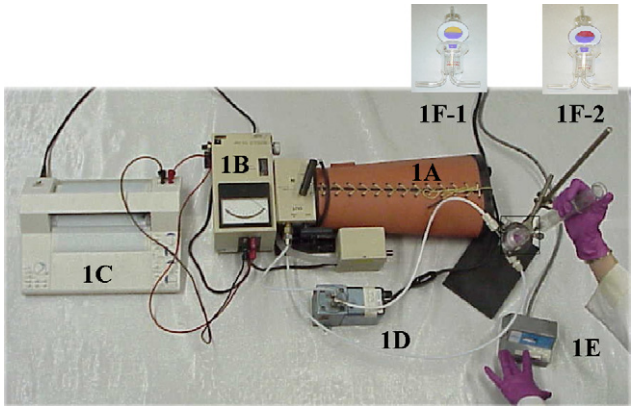


Fig. 1. A Miran (Miniature Infrared Analyzer) closed-loop configuration: Miran (A) with its IR detector (B); a chart recorder (C); metal bellows pump (D); timer (E); and a 2.5-cm chemical permeation cell (indicator sensor pad with a yellow color (F-1); on exposure to glutaraldehyde, the pad changed color to red color (F-2).

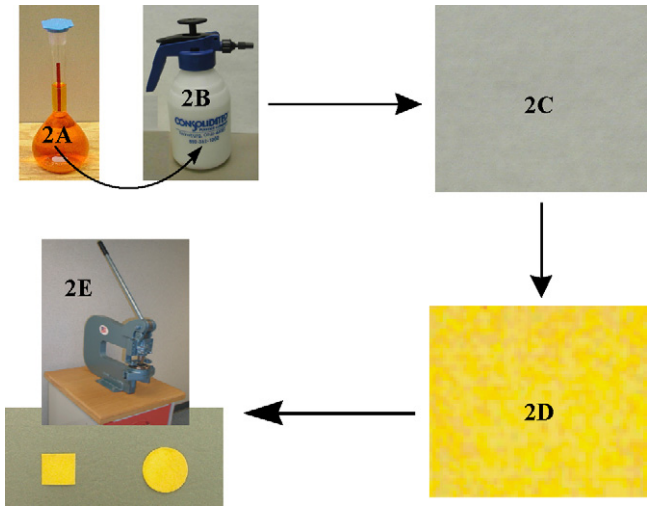


Fig. 2. Aldehyde indicator in the solid phase: aldehyde indicator (A); a hand-operated sprayer (B); the pad material sheet (C); the indicator sheet (D); a hand operated puncher (E).

2.2.2. Indicator pad fabrication

The aldehyde indicator solution (Fig. 2A) was applied at a concentration of 27 μg/cm^2 onto the absorbency side of the new pad materials (cellulose) either by using a hand-operated sprayer (Fig. 2B) or by dipping the pad-material sheet (Fig. 2C) into the indicator solution. The wet pad materials, containing an aldehyde indicator (referred to as indicator sheets), were dried under a hood at room temperature for 24 h. Then, the indicator sheets (Fig. 2D), were dried at 65 °C for 6 h using an Isotemp Oven to remove residual water and methanol from the indicator sheets. The polyethylene in the backing side of the indicator sheet was then taken off and the indicator sheets were dried at 65 °C for another 2 h before being used for pad fabrication. The backing side of the indicator sheet was then coated with a double-sided plastic tape (www.DuckProducts.com, Cat. #: DT-75-1.88 in. ×75 ft) to produce the sheets suitable for fabricating the indicator pads. These indicator sheets were cut using a hand-operated puncher (Fig. 2E) and attached to a nonsterile adhesive

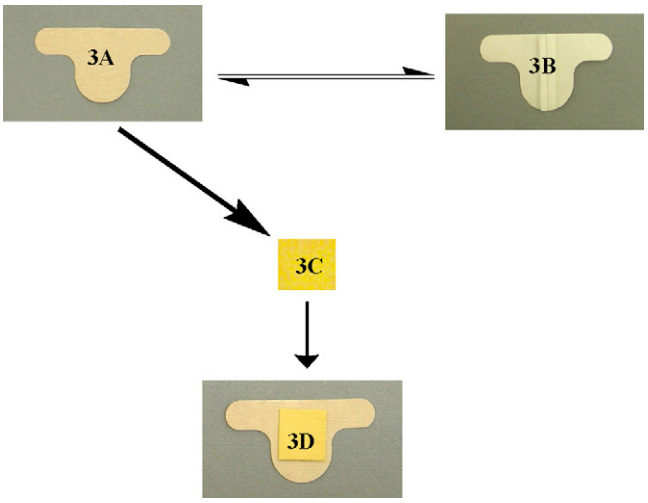


Fig. 3. Design and fabricate new aldehyde indicator pads: top side of adhesive strip (A); bottom peel-pouch side of strip (B); aldehyde indicator sensor (C); a complete aldehyde indicator pad (D).

bandage (Fig. 3, www.buymed.com, BEI00801) before being used.

2.3. The sensitivity study for aldehyde indicator pads

2.3.1. Testing the indicator with liquid aldehydes

The sensitivity of the indicator pads for aldehydes in the liquid phase was conducted by spiking chemicals directly onto the surface of the indicator pads. The amount of aldehydes required to produce a noticeable color change was determined by spiking a known standard aldehyde directly to the surface of the indicator pads using a calibrated syringe. A timer was immediately started upon the application of the spike.

2.3.2. Testing the indicator with vapor aldehydes

A sensitivity assessment of the indicator pads for aldehydes in the vapor phase was run according to the modified ASTM F739 method [24]. The Miran-IA instrumental settings were as follows: slit, 1.0 mm; wavelength, 3.7 μm; pathlength, 20.25 m; and minimum detectable concentration, 1 ppm for glutaraldehyde [25]. The 2.5-cm permeation cell is divided into a liquid phase "challenge side" which contains 50% glutaraldehyde in water, and a vapor phase "collection side" which contains the sweep gas (house air in a closed loop; flow rate, 11.328 L min^{-1}). A glove membrane (sections from the palm of the gloves) separated the two sides of the permeation cell, with the outer surface toward the challenge side of the permeation cell. A half circle of an indicator pad (Fig. 1F-1) was attached to half of the inner surface of the glove section and covered with clear plastic tape. The other half of the glove section was left unobstructed so that permeating glutaraldehyde could reach the analyzer detector. This system was operated in the closed-loop mode and the experiment was conducted at room temperature (22 ± 1 °C). 15 mL of 50% glutaraldehyde solution was injected into the challenge side of the cell using a 30-mL glass syringe, and a timer and a metal circulation pump were immediately started. Permeation of glutaraldehyde through the glove was collected and subsequently detected by either the change in color of the pads or the infrared analyzer response.

2.4. Chemical principle and characterization of the test

The chemical reaction, interaction mechanism and its relationships to color change were characterized using pH data, absorption spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy.

2.4.1. The relationships between color-formation and pH

The color and the pH relationships of indicator solutions and products of glutaraldehyde solutions and indicators (referred to as reaction products) were characterized visually and using a pH meter. The color change of indicator pads was also characterized by spiking glutaraldehyde, alkaline glutaraldehyde, and non-alpha hydrogen aldehyde solutions (1.0–3.0 μL) in the pH range from 4.55 to 8.63 onto the surface of the indicator pads using a syringe.

2.4.2. Absorbance spectroscopy

The UV–vis (ultraviolet visible) spectra of the indicator and the reaction product in deionized H_2O were obtained at room temperature using an U-3010 module spectrophotometer (Model UV–Vis 3010, Hitachi, www.hii.hitachi.com).

2.4.3. NMR spectroscopy

All NMR sample concentrations were the same as stock samples as described in the "Aldehyde indicator solution preparation" section without the presence of methanol. All NMR indicator samples contained 1.2 mM indicator in 0.5 mL of 90% D_2O and 10% H_2O/glycerine. The NMR reaction product samples contained 1.2 mM indicator in the presence of glutaraldehyde (the ratio of glutaraldehyde/indicator = 1/1 to 2/1 in mole) in 0.5 mL of 90% D_2O and 10% H_2O/glycerine. The samples in D_2O were prepared by lyophilization of the water samples and resuspension in D_2O. ^1H NMR spectra were recorded at 599.672 MHz on a Varian INOVA 600 spectrometer at 298 K. Data were processed using Varian software (MSI). Water suppression was achieved through presaturation of the H_2O signal during the relaxation delay. TPPI (time proportional phase incrementation) was used for performing phase-sensitive 1D and 2D NMR experiments. All spectra were referenced to the chemical shift of the residual HDO signal at 4.85 ppm (relative to TMS). In general, 64 transients were recorded with a relaxation delay of 2–3 s and a spectral width of 8.1 kHz. 512 increments of 2 K data points were collected in each 2D COSY experiment and were zero filled so that spectra with 2 k × 2 k data points were obtained.

2.5. The interference study for aldehyde indicator pads

Volumes of 1.0–3.0 μL of chemicals containing different functional groups were spiked directly onto the surface of the indicator pads using a syringe. A timer was immediately started upon the application of the spike. Based on the indicator structure, some chemical groups were selected for testing

for chemical interference, including organic and inorganic acids (HCl, H_2SO_4, acetic acid, and acrylic acid), organic and inorganic bases (NH_4OH, NaOH, KOH, and butyl amine), alcohols (ethanol and 1-octanol), and ketones (acetone and 2-butanone).

3. Results

An aldehyde indicator was developed and a new indicator pad was fabricated for detecting aldehyde exposures (Fig. 3D). It was shown that the indicator pad was sensitive enough to detect 5 μg of glutaraldehyde (0.5 μL of 1% glutaraldehyde in water) spiked onto the pad. Glutaraldehyde caused the pads to change from yellow to red immediately (within a second). The aldehyde pad which carries thereon a predetermined reagent was designed to be responsive to contact by an aldehyde or aldehydes to produce a visible color indication; therefore, the reuse of this indicator pad is not recommended. The minimum detectable concentration of glutaraldehyde required to produce a noticeable color change was also determined and found that when ≤0.5 μg of glutaraldehyde (0.5 μL of 0.1% glutaraldehyde in water) was spiked directly to the surface of the pads, the color of the pads changed from yellow to orange-red, but it disappeared within a minute. With the ASTM F739 permeation method, a significant visible color change from yellow to red occurred on the pad about 5 min before the infrared analyzer responded. Therefore, the determination of the breakthrough time of glutaraldehyde through the gloves was highly dependent on the sensitivity of the indicator detection method with the indicator pad response being faster than the vapor phase infrared analyzer.

It was also shown that the indicator pad was sensitive to detect alkaline glutaraldehyde and non-alpha hydrogen aldehyde solutions. The aldehyde indicator formed a red or orange-red color in contact with alkaline glutaraldehyde solutions in the pH range from 4.55 to 8.06 (Table 1). However, alkaline glutaraldehyde solutions with pH > 8.06 remained yellow (Table 1). The aldehyde indicator also formed a red color in contact with formaldehyde and benzaldehyde solutions (≥8% formaldehyde solution in water and ≥5% benzaldehyde solution in cyclohexane).

Methanol was determined by a gas chromatography (GC) analysis following the solvent extraction process as described by Vo [14]. The indicator showed the absence of the methanol peak on a GC chromatogram when a new aldehyde indicator pad (1.8 cm square pad, Fig. 3C) has been extracted in 300 μL of distilled water, and volumes of 5 μL of extracted samples were injected into the GC column using a syringe. This result indicates

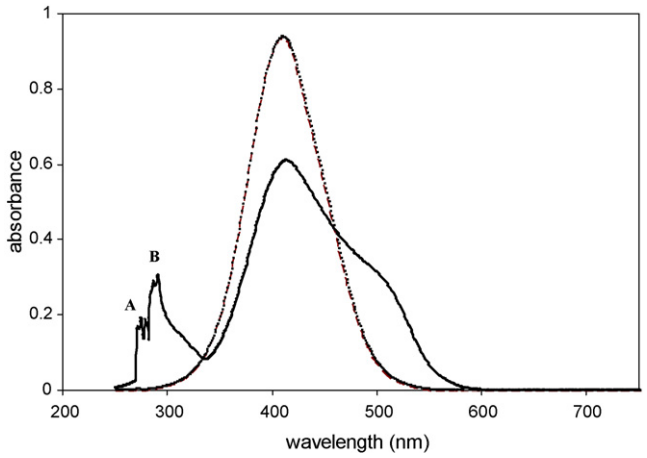


Fig. 4. Absorbance spectra of indicator (dashed line) and the reaction product between glutaraldehyde and the indicator (solid line) at room temperature.

that the methanol used to spread glycerine on the surface of the pad materials was removed from indicator pads. The indicator color was very stable in the solid phase of the pad (without glycerine, the indicator color was not stable at room temperature, as it changed from yellow to black). The thermal stability of the indicator pad was also monitored at 65 °C for 8 h (Note: these pads were tested with glutaraldehyde after storing them inside an aluminum bag at room temperature for 1 year, and changed color from yellow to red, and thus the pads are expected to have a minimum of 1 year of shelf-life).

The color and pH changes occurring with the reaction between glutaraldehyde and the indicator were characterized. The orange-red color of the reaction product formed when adding 0.15 mL of glutaraldehyde into the 500-mL indicator at pH = 7.85. With sufficient glutaraldehyde added into the indicator (≥0.2 mL of glutaraldehyde per 500 mL of the indicator solution), the reaction product changed to a red color and its pH decreased to the neutral pH.

The UV–vis spectrum of the reaction product (Fig. 4, solid line) was essentially identical to that of the indicator in the UV–vis region of 300–520 nm (Fig. 4, dashed line). The absorbance bands in the UV–vis region of 300–520 nm indicated a significant complex structure of 2-[4-dimethylamino)phenylazo]benzoate anion. Furthermore, the spectrum of the reaction product with two additional bands (a weak band in the region of 270–320 nm and a strong shoulder band in the region of 460–570 nm) was distinct from that of the indicator. A weak band (containing 2 peaks: A and B; Fig. 4) in the ultraviolet region, ranging from 270–320 nm with λ_{max} at

Table 1
The color-formation data of the reaction between glutaraldehyde solutions and indicators

Testing glutaraldehyde solutions	Amount of glutaraldehyde in solutions (spiking volumes to pad)	The pH of glutaraldehyde solutions	Positive detection (original indicator color: yellow)	Negative detection
Glutaraldehyde	2.0% glutaraldehyde in water (1 μL)	4.55	Red color was formed	
Wavicide®	2.65% glutaraldehyde in inert ingredients (1 μL)	6.20	Red color was formed	
Metricide®	2.5% glutaraldehyde in other ingredients (2 μL)	7.92	Red color was formed	
Procide-D®	2.5% glutaraldehyde in other ingredients (3 μL)	8.06	Orange-red color was formed	
Cidex®	2.4% glutaraldehyde in other ingredients (3 μL)	8.63		No color was formed

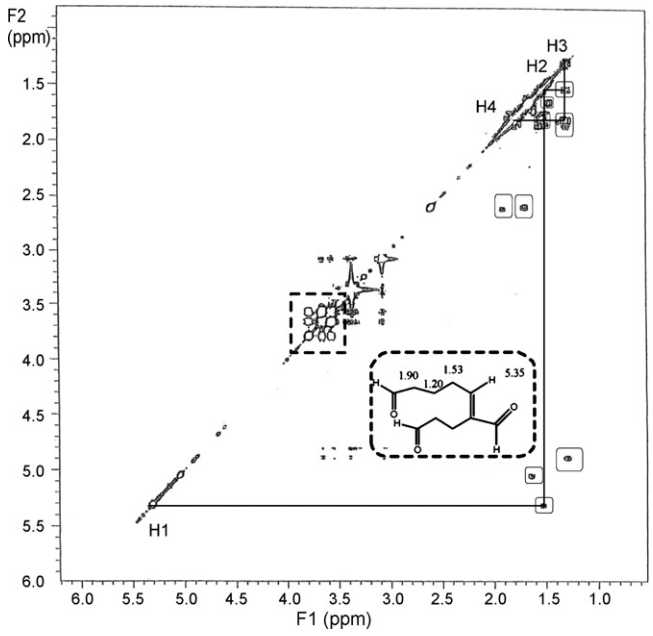


Fig. 5. Two-dimensional DQF-COSY spectra of indicator (above diagonal) and the reaction product (below diagonal) taken at 298 K. The samples of both spectra were 0.5 mL of 1.2 mM of indicator and the reaction product in 90% D_2O and 10% H_2O/glycerine. Identical cross peaks of the indicator and the reaction product spectra in the region of the 3.5–3.8 ppm are boxed (dashed line box). New cross peaks that were detectable in the reaction product spectrum in the region of 1.20–5.35 ppm are boxed (solid line box) and labeled according to sequential assignment.

275 and 290 nm, indicated non-conjugated and conjugated aldehyde groups, respectively. A strong shoulder band in the visible region, ranging from 460–570 nm, is related to the extended conjugation with a delocalized positive charge in the azo complex structure which is suggestive of the observed color change from yellow to red [26,27].

The structural features of the reaction product were assessed by NMR spectroscopy. All ^1H–^1H cross peaks in the COSY spectra of the reaction product had a counterpart at an essentially identical position in the spectra of the indicator in the region of the 3.5–3.8 ppm (Fig. 5) and 6.8–8.0 ppm. The signals in the region of the 3.5–3.8 ppm were assigned to glycerol protons while the signals in the region of the 6.8–8.0 ppm were assigned to phenyl protons of 2-[4-dimethylamino)phenylazo]benzoate anion. Several differences in the appearance of the spectra of the indicator and the reaction product, however, were apparent. The reaction product spectrum displayed some additional resonances of aldehyde functional groups in the region of 9.6–9.9 ppm and the glutaraldehyde polymer main-chain peaks in the region of the 1.20–5.35 ppm (Fig. 5). Convincing evidence for the presence of the glutaraldehyde oligomer in the reaction product was obtained from the COSY spectrum (Fig. 5). The sequential assignment for the glutaraldehyde oligomer was started by searching for alkenyl protons ($R_2C=CHR$), and found three correlation peaks with roughly equivalent intensities at 5.35, 5.05, and 4.90 ppm (Fig. 5). The sequential assignment was continued from each proton peak and a next aliphatic alkyl proton ($R_2C=CH–CH_2R$). A detailed example of the sequential assignment for the glutaraldehyde dimer segment was started from a proton at 5.35 ppm (H1 of $R_2C=CHR$, Fig. 5). This proton peak correlated to a next aliphatic proton at 1.53 ppm (H2 of $R_2C=CH–CH_2R$, Fig. 5). The aliphatic H2-proton peak correlated to a H3 proton at 1.20 ppm ($R_2C=CHCH_2CH_2R$, Fig. 5). This sequential assignment was ended at a H4-proton adjacent to a carbonyl of aldehyde group at 1.92 ppm ($R_2C=CHCH_2CH_2CH_2COH$).

The interference tests were performed for organic/inorganic acids, organic/inorganic bases, alcohols, and ketones. The results indicated that only acids interfered with the aldehyde indicator, while none of the organic/inorganic bases, alcohols, and ketones formed color when used neat or in solution.

4. Discussion

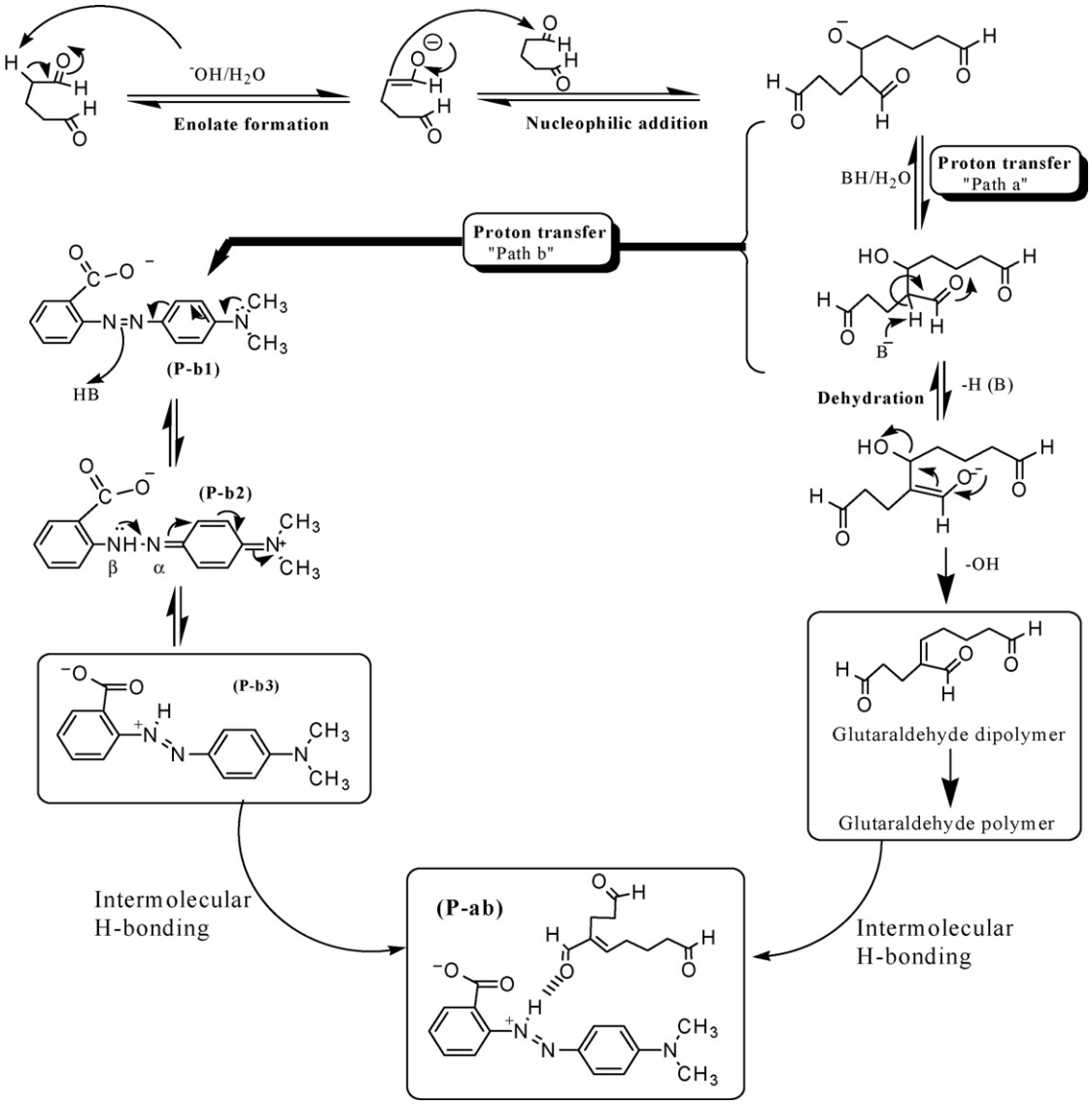
The indicator-glycerine complex in the cellulose pad material afforded a stable product that would incorporate hydrogen-bond elements between the indicator-glyceryl complex and the polysaccharide of the pad materials. The slowly exchanging NH and OHs which were believed to be possibly involved in hydrogen bonds will be further investigated in our future study using the same methods of Vo et al. [28] and Mino et al. [29]. Indicator solutions applied to pads have a very low vapor pressure and low concentration (about 27 $\mu g/cm^2$). The methyl red reagent was tested by NIOSH's Health Effects Laboratory using the Local Lymph Node Assay and phenotypic analysis and was found to be a contact sensitizer [30]. To protect against skin exposure, the reverse side of the indicator pad is covered with an impermeable double-side plastic tape.

The indicator was sensitive enough to detect 5 μg of glutaraldehyde applied to the pads. In the vapor phase, glutaraldehyde and glutaraldehyde solutions were detected by the change in the color of the indicator pad before the infrared analyzer responded.

The UV–vis spectrum of the reaction product revealed the conjugated aldehyde group and the extended conjugation with a delocalized positive charge in the azo complex structure. The extended conjugation of the azo complex structure would yield a longer wavelength of visible light and the observed color change from yellow to red.

The structural features of the indicator and the reaction product were assessed by NMR spectroscopy. The COSY spectrum of the reaction product displayed new peaks of aldehyde functional groups in the region of 9.6–9.9 ppm and the main chain of the glutaraldehyde polymer in the region of the 1.20–5.35 ppm. The sequential assignment for the glutaraldehyde polymer was convincing evidence that the oligomer was formed.

Under the basic conditions employed, glutaraldehyde would rapidly form an enolate ion which acted as a nucleophilic carbon to attack the carbonyl group of another molecule of glutaraldehyde. This self-aldol condensation resulted in the formation of glutaraldehyde oligomers (Scheme 1, Path a). When the pH of the reaction product decreased to about neutral pH, the 2-[4-dimethylamino)phenylazo]benzoate anion with two electron-donating methyl groups at the terminal amino group in the *para* position was able to delocalize the lone pair of elec-



Scheme 1. Chemical reaction and mechanism between glutaraldehyde and the indicator under a base-catalyzed condition.

trons into the π-system (P-b1 in "Path b", Scheme 1). Therefore, the protonation of the 2-[4-dimethylamino)phenylazo]benzoate anion occurred to a greater extent on the β-nitrogen atom of the azo group (P-b3 in "Path b", Scheme 1) to yield the azonium tautomer with both cationic and anionic groups (P-b3 in "Path b", Scheme 1). This tautomer contained a delocalized positive charge (P-b2 ↔ P-b3) [26,31]. The azo proton in the azonium tautomer (P-b3) would form a hydrogen bond to the carbonyl group of conjugated aldehyde in the azo-complex compound (P-ab) or to the oxygen in the anionic group to form a 6-membered chelate ring [26] to increase the exceptional stability of the azo-complex compound. The azo-complex compound containing a delocalized positive charge form caused the color change in the indicator from yellow to red [26,27].

In solution studies, glutaraldehyde would undergo self-aldol condensation to yield the glutaraldehyde oligomers, and the orange-red color of the reaction product formed at pH = 7.85 suggests this is due to the color of the indicator glyceryl complex. It is probable that with the low aldehyde concentration, the pH of the indicator decreased to the range of $7.0 < pH \leq 8.06$, and the orange-red color appeared due to the significant van der Waals forces between indicator and glycerine. These forces in the aldehyde indicator are not as strong as H-bond forces, but they play an important role in the physical properties, such as boiling point, solubility, and color of the indicator-glyceryl complex compound. With sufficient glutaraldehyde added into the indicator, the reaction product changed to a red color and the pH decreased to a neutral pH. A possible explanation for the decreased pH would be the acidic nature of glutaraldehyde and a significant amount of Cannizarro reaction [32] forming a carboxylate salt and an alcohol from two glutaraldehydes. At about neutral pH, the protonation of the 2-[4-dimethylamino)phenylazo]benzoate anion occurred to a greater extent on the β-nitrogen atom of the azo group to yield the azonium tautomer with both the β-nitrogen cationic and carbonyl anionic groups. The azo proton in the azonium tautomer would form a hydrogen bond to the carbonyl

group of conjugated aldehyde in the azo-complex compound to increase the exceptional stability of the azo-complex compound. The azo-complex compound containing a delocalized positive charge form caused the color change in the indicator from yellow to red [26,27]. However, when the pH of the indicator solution decreased to pH < 7.0, the protonation of the 2-[4-dimethylamino)phenylazo]benzoate anion occurred both on the β-nitrogen atom and carbonyl anionic groups to form an acid. Even though this azonium tautomer contained both cationic groups, it would have the same color characteristic as the azonium tautomer (P-ab, Scheme 1) because the positive charge on β-nitrogen atom only contributed a delocalized positive charge in the conjugated indicator system.

Non-alpha hydrogen aldehydes, such as formaldehyde (≥8% formaldehyde solution) and benzaldehyde (≥5% benzaldehyde solution) also changed the color of the indicator pad from yellow to red, but the indicator pads were less sensitive to their detection compared with glutaraldehyde solutions (1% glutaraldehyde solution). A possible explanation for the low response for these non-alpha hydrogen aldehydes is that these aldehydes would only undergo of Cannizarro reaction forming a carboxylate salt and an alcohol from two non-alpha hydrogen aldehydes to decrease pH in the indicator pads; however, these aldehydes did not undego self-aldol condensation to yield the aldehyde oligomers which would contribute an extended conjugation in the azo-complex compound.

The indicator formed a red or orange-red color in contact with glutaraldehyde solutions in the pH range from 4.55 to 8.0. Interestingly, a 2-[4-dimethylamino)phenylazo]benzoic acid, sodium-salt, reagent in alcoholic solutions or in water is associated with a change in pH [the change in color of methyl red is associated with a change in pH in alcoholic solutions at pH 4.4 (pink-red) and at 6.2 (yellow) as described in *Aldrich Catalog*, 2003/04, p. 1287]. Therefore, the new aldehyde indicator forming a red or orange-red color in contact with glutaraldehyde solutions at $4.40 < pH < 8.06$ appears to be uniquely associated with the reaction of aldehydes and the indicator to detect aldehydes outside the range of the normal use of the 2-[4-dimethylamino)phenylazo]benzoic acid, sodium salt, reagent as a pH indicator.

5. Conclusion

An indicator pad was developed for detecting aldehydes. It was shown that the new indicator pad responded to glutaraldehyde with a visible color change from yellow to red about 5 min before the infrared analyzer responded. This indicator pad can be used to determine glove permeation to glutaraldehyde and alkaline glutaraldehyde solutions. The color formation of the indicator in contact with glutaraldehyde solutions over the range $4.40 < pH < 8.06$ appears to be associated with the reaction and interaction of aldehydes and the indicator. The aldehyde indicator should find utility in detecting aldehyde solutions in the pH range from 4.40 to 8.06, in which the normal pH indicator of 2-[4-dimethylamino)phenylazo]benzoic acid reagent does not respond.

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Disclaimer: The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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